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Agricultural Research



Research to This issue of Agricultural Save Lives

Research features a story on ways to control the dust that comes from

corn, wheat, and other grains as they are lifted, conveyed, and dropped by the nation's 15,000 elevators on the way to becoming food or feed.

Although controlling dust may sound more like an esthetic matter, it is actually an important safety problem. Dust has a tremendous amount of potential energy, and under certain conditions, it can detonate with explosive force.

The danger inherent in grain dust came sharply into national focus nearly 8 years ago when in 8 days -during the 1977 Christmas holiday season—5 explosions took 59 lives and injured 48.

Fortunately, fewer explosions have occurred since then thanks to a far-reaching research and training effort undertaken by industry, government, and educational institutions. The grain industry itself has been a major contributor to the scientific investigation of elevator accidents.

The aim of research on fire and explosion prevention has been to develop procedures that elevators can use to avoid the factors that must be physically present before an explosion can occur. These factors are: dust, oxygen, confinement, and an ignition source.

Logically, one way to positively avoid dust explosions would be to prevent the rapidly burnable dust from accumulating in any place where the other three elements of an explosion could be present.

One of the most promising methods of dust suppression (and one requiring minimal capital investment to put into use) is coating grain kernels with a minute film of food-grade oil as they pass through the grain-handling equipment. The kernels then attract and hold dust particles during the rest of their trip through the elevator.

Addition of mineral oil to grain has Food and Drug Administration approval at levels of 200 parts

per million (ppm) for food uses and 600 ppm for animal feed. Researchers are also looking at edible oils such as soybean oil as a dust prevention additive. The question of whether the oil changes the grade of grain or affects baking quality is being studied.

Since dust is composed largely of starch, it retains its feed value to livestock. ARS researchers are checking for economical ways to remove the dust from the grain as it is moved through the elevator and market it.

It is not practical to try to eliminate oxygen from possible points of ignition in a grain elevator, but systems have been designed to smother flames in certain confined areas of an elevator after a fire has started.

As more is learned about the movement of fire and explosions through an elevator, designs for new and remodeled installations have reduced the number of confined spaces. In cases where confinement is necessary around equipment, for example, vents are being added to channel the force of a possible explosion harmlessly into the outside air. At Westwego, LA, a grain-handling facility with a new design has been built to replace an elevator destroyed in one of the Christmas-week explosions of 1977. Its construction has eliminated confined spaces at the top of the silos and in the internal vertical-grain-elevating equipment in favor of overhead distribution pipes and sloped conveyors outside the building.

Total elimination of ignition sources is often difficult near moving machinery commonly found in grain elevators. Conveyor belts out of alignment might rub until a fire starts, or a bearing could overheat. Welding sparks could start a fire if not sufficiently protected. However, redesign of machinery and structures and careful maintenance has been shown to greatly reduce chances of igniting dust. Industryfinanced research has indicated that two other suspected causes-static electricity and sparks from metal striking metal or concrete—are unlikely sources of ignition.

> Lloyd McLaughlin Beltsville, MD



Agricultural Research

COVER: Grain elevators, a familiar sight throughout the country, are becoming safer because of research on causes and ways to prevent dust explosions. Story begins on page 6. (0985X1052-4)



Trapping the Explosive Grain Dust

Scientists hope to prevent explosions.

10 Ion Channels In Plants Work Like Simple **Nervous System**

Discovery may open new avenues to controlling plant growth and yield.

12 Aflatoxin — No One Solution

Weather stresses and farming practices may weaken plant defenses against toxin-producing molds.





Leaner Lamb Expected From Texel Sheep Genetic Engineers Study Crop Growth Control Turning Surplus Fats and Oils into Useful Ones "Wimp" Mold May Save Grain From Bad Guys



Herbicide-Coated Seeds Pheromone For Grain Weevils **Bacterial Protein Extraction Process**

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Leaner Lamb Expected From Texel Sheep

A white-faced meaty sheep, native to the Netherlands, valued for its lean meat, will be bred in this country for the first time by USDA's Agricultural Research Service.

The breed, Texel, recently arrived in the United States from Finland and Denmark, countries free of foot-and-mouth disease. "We are studying Texel sheep for any possible help in increasing the efficiency and quality of U.S. lamb production," says Robert R. Oltjen, director of the U.S. Meat Animal Research Center, Clay Center, NE. Texel's growth rate, feed requirements, and survival rate of newborn will also be investigated at Clay Center.

One possibility could be the Texel becoming a new source of lean lamb for consumers, he says. That could come either from breeding the sheep itself or from

Texel sheep in quarantine at the U.S. Meat Animal Research Center, Clay Center, NE. (0985X1036-32)

crossing it with domestic sheep.

Any impact that the Texel may have on the lamb industry will be a number of years away, Oltjen says. All of the new sheep, totaling 4 rams, 20 ewes, and 31 lambs born enroute and after arriving at the Center will be quarantined to a 20-acre site for the next 5 years.

USDA's Animal and Plant Health Inspection Service established the quarantine until the Texel can be certified free of scrapies, a debilitating disease of sheep brain tissue. Scrapies often takes several years to show up in flocks. ARS has taken elaborate precautions to keep the Texel isolated at Clay Center and yet make it possible for researchers to undertake studies.

Imports by the U.S. sheep industry have been prohibited because of concern about scrapies.

Texel is the first sheep breed to be imported from Europe since the 1960's, Oltjen says. At that time, USDA brought in another breed, Finnsheep, for various studies, including one of its high birth rate.

As part of the current research, animal scientists will compare Texel lambs with those from domestic Suffolk rams and half-Finnsheep ewes for lamb survival, growth rates, carcass leanness and other characteristics, says Center geneticist Kreg A. Leymaster.—Ben Hardin, Peoria, IL.

Robert R. Oltjen is at the USDA-ARS Roman L. Hruska U.S. Meat Animal Research Center, P.O. Box 166, Clay Center, NE 68933. ■

Genetic Engineers Study Crop Growth

Plant breeders may someday be able to postpone aging of crops, giving plants extra time to grow. The result—increased yields and better quality. Research chemist Joseph W. Corse and other Agricultural Research Service scientists in Albany, CA, are determining how to genetically modify plant growth hormones and the enzymes that control the hormones. He gives an example of what might be achieved: Manipulating enzymes to delay aging in wheat would give the plant more time to add protein to seeds.

The research focuses on three classes of compounds that occur naturally in plants and are the most promising targets for genetic manipulation—gibberellins, cytokinins, and ribonucleases.

Gibberellins and cytokinins are plant hormones with important characteristics in common—they affect growth, and they're controlled by enzymes.

But more needs to be discovered about these powerful hormones before they can be controlled. An example: It's already been shown that two kinds of gibberellin occur in wheat leaves. But are there others? If so, what enzymes control their concentrations? Once this information is known, the scientists should be able to change the hormone concentrations by genetic engineering of the controlling enzymes.

The cytokinins are "much like gibberellins in that they are very complex chemically," says Corse. Despite this complexity, chances of success with the cytokinin research are high, because cytokinins have already proven their ability (in other experiments) to counteract aging.

"We need to find out the concentrations of all forms of cytokinins in wheat—and similar crops—and to learn how these concentrations vary during different periods of growth," Corse explains. "Only when we have this information can we then begin to genetically modify the enzymes that control cytokinins."

Ribonucleases—the other compounds of interest—are

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enzymes that can change the amount of other growth-related enzymes available to a plant.

As with the studies of the hormones, much effort is going into answering such basic questions as how many ribonucleases occur in wheat leaves? So far, the Albany researchers have discovered 11. Of these, 9 are associated with aging.

Corse gives this perspective on the research: "We're not out to make plants live forever. we just want to change things a little. Plants already have built-in mechanisms to regulate hormone concentrations. It's these control mechanisms we're after."—

Marcia Wood, Albany, CA

Joseph W. Corse is at the USDA-ARS Western Regional Research Center, 800 Buchanan St., Albany, CA 94710. ■

Turning Surplus Fats and Oils Into Useful Ones

Enzymes may hold the key to transforming fats and oils that are plentiful into imitations of currently scarce or expensive ones.

Enzymes such as lipases, giant molecules that are generated by living things to degrade the fats and oils they ingest, also have the ability to turn one kind of fat into another.

If scientists knew how these enzymes work, they might be able to turn an inexpensive fat such as beef tallow into one with the properties of a more valuable fat, say cocoa butter.

To study such enzymes, Agricultural Research Service scientists hope to produce and isolate a lipase enzyme from bacteria, using modern techniques of genetic engineering.

Experiments are also underway using commercial enzyme preparations made from fungi to

study the structure and performance of lipases.

The scientists leading this research, chemists Philip E. Sonnet and Michael J. Haas, are at the USDA-ARS Eastern Regional Research Center, 600 East Mermaid Lane, Philadelphia, PA 19118.

"Wimp" Mold May Save Grain From Bad Guys

Farmers generally consider molds bad, but there is one mold that may be good when it comes to grain storage.

David B. Sauer, an Agricultural Research Service plant pathologist, calls the good mold, Aspergillus glaucus, a "wimp" because it grows slowly and lacks aggressiveness. "Indeed, A. glaucus passively does its own thing, which turns out to be important because it limits the effects of other, more damaging molds," Sauer says.

Sauer is studying competition among molds and looking for an effective, nonchemical way to reduce fungal growth and damage in stored grain at the U.S. Grain Marketing Research Laboratory in Manhattan, KS. Sauer inoculated corn and wheat samples with three strains of *A. glaucus* and a combination of the five most damaging molds.

Fungal growth was measured by how much crystalline steroid alcohol (ergosterol) the molds produced. "If grain is clean and fungus-free, there will be an absence of ergosterol. As molds grow on grain, the amount of ergosterol increases," says Sauer.

Tests were made at 68°F and above, at a relative humidity of 85 to 90 percent.

Inoculation with A. glaucus plus the five species resulted in less total fungal growth than with just the five species.

In a 1979 survey of export



Plant pathologist David Sauer examines corn samples to determine total fungal growth after inoculation with *Aspergillus glaucus* and a combination of the five molds most damaging to stored grain. (0785X640-20A)

grain samples, Sauer found mold in every corn sample. "On the average, about 20 percent of the kernels had storage mold in them, which is probably typical of corn in commercial channels," he says.

Sauer says the storage environment is responsible for fungus invasion and growth. Such factors as temperature, ventilation, and moisture are critical in the growth of storage molds.

"Storage molds are everywhere—so we really can't avoid grain contamination. Studying the effects of *A. glaucus* on the more damaging molds may help us to better understand and control grain spoilage," says Sauer.—Linda Cooke-Stinson, Peoria, IL.

David B. Sauer is in USDA-ARS Biological Research at the U.S. Grain Marketing Research Laboratory, 1515 College Ave., Manhattan, KS 66502.

Trapping the Explosive Grain Dust

In studies to determine conditions that create explosions in elevators, chemical engineer Fang Lai produces small-scale explosions in a 20-liter combustion chamber. The instruments attached to chamber measure concentrations of grain dust and explosion pressure. (0785X630-11A)

Traces of soybean oil may help prevent grain elevator explosions.

In his latest research, chemical engineer Fang S. Lai at the U.S. Grain Marketing Research Laboratory in Manhattan, KS, has found that coating grain kernels with equal amounts of soybean oil and lecithin may be a good way to keep grain dust out of the air. Lecithin is an inexpensive byproduct of soybean oil processing.

The sticky coating traps dust and holds it on the kernels. This is important in preventing explosions because it is the airborne dust that explodes when triggered by heat or flame.

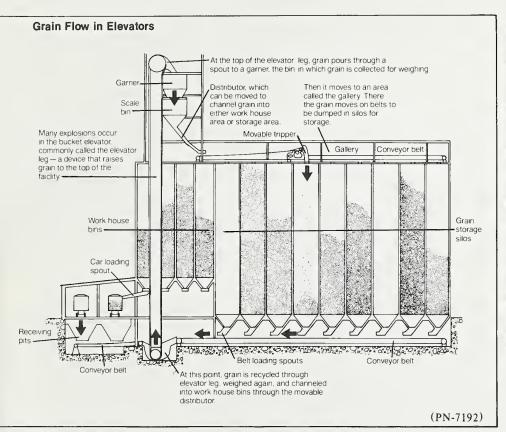
No one knows for sure the ignition source for many of the explosions in the past, because often "the reason for an explosion goes up with the rest of the building," in the words of one grain handler. Likely sources of ignition include overheated bearings, slipping belts, faulty motors, and sparks from welding.

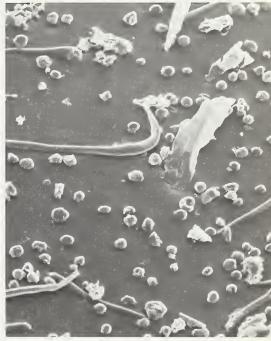
Lai says the dust that fuels grain elevator explosions is "generated whenever the mechanical stress of handling grain causes kernels to crack."

The fine dust is ground from the starch inside kernels broken somewhere in the handling, from harvest to storage. Lai says although all grains produce dust, corn creates the most because it cracks easier.

This "dust gathers on everything in an elevator enclosure from the ceiling to the floor, and totally eliminating it is impossible," says Lai.

To learn more about what happens to kernels as grain is poured into silos, Byron S. Miller, at the time a chemist at the grain lab, now retired, designed a device that hurls kernels down a 1-meter drop (39 inches) to simulate the impact of a 100-foot fall. With high-speed photography, the scientists can see what happens as kernels strike a bed of





Scanning electron micrograph shows wheat dust particles obtained from a cyclone collector in a grain elevator. Such micrographs aid researchers in studying the physical characteristics of dust. (PN-4181)

grain and bounce, setting other kernels in motion and producing dust particles.

Grain insects contribute a little to the dust both while they feed on kernels and when they die and their bodies disintegrate.

Lai and his colleagues are trying to find ways to keep dust out of the air. One method under study is to coat the grain with edible vegetable or mineral oils, or water so dust will stick to the kernels.

Lai says oils may be better than water. Depending on the moisture content of the grain, water would have to be applied each time the grain is handled, and too much water could cause the grain to mold. Oil will not cause grain to mold and is still significantly effective after 3 months' storage.

Agricultural engineer Charles R. Martin, a colleague of Lai's, says it's critical to mix grain thoroughly after applying oil or water.

Martin says, "We found that dust suppressants were best applied by continuously spraying both the top and the underside of the grain stream" at the belt-loading spout as the grain leaves the storage silo.

In cooperative research with the American Soybean Association and the National Grain and Feed Association at an Ohio grain elevator, Lai and coworkers found that adding 500 parts per million (ppm) soybean or mineral oil to grain cuts dust by 90 percent. They found that little oil from the treated grain spread to equipment or walls.

In related research on treating grains with oil, Vernon L. Youngs, ARS food technologist at Fargo, ND, found no significant differences in milling and baking quality of the flour. He sprayed mineral oil on

wheat at approximately 1-month intervals until 800 ppm had been added to simulate the multiple oil treatments a grain might receive in a marketing chain.

Using suppressants is one way to control dust, but many elevators may want to actually remove it with bag filters, electronic precipitators, and cyclone collectors that separate dust from the air.

The costs of such dust removal equipment can be partly offset by recycling the dust collected. Cheng S. Chang, also an agricultural engineer at the Grain Marketing Research Laboratory says, "It can be pelleted or extruded as nutritious cattle feed, used as fuel for drying grain, or composted for organic soil conditioner or fertilizer." Chang found that composted grain dust contains 2.5 percent nitrogen, 0.5 percent phosphorus, and 1.7 percent potassium.

What Else Can Be Done?

To find out what initiates, triggers, and propagates grain dust explosions, Lai and coworkers produce small-scale explosions in a 20-liter chamber.

With this device they test the size of sparks needed to ignite dust, how much explosive pressure is created, how fast pressure rises, and how much dust must be in the air before it explodes.

In another dust-control project, Chang and coworkers at Manhattan designed a grain flow regulator that keeps the dust within the grain stream as it flows into a silo. Their test results show that the flow regulator reduces dust emission by 20 percent compared with dust from grain passed through a conventional nozzle.

"We cannot avoid having some dust," Lai says, "but with proper control, based on sound research, the risks of dust explosions can be minimized."—Betty Solomon, Peoria, IL.

Fang S. Lai, Cheng S. Chang, and Charles R. Martin are in USDA-ARS Engineering Research at the U.S. Grain Marketing Research Laboratory, 1515 College Ave., Manhattan, KS 66502. Vernon L. Youngs leads USDA-ARS Spring and Durum Wheat Quality Research at North Dakota State University, Fargo, ND 58105.



Burning grain sent black smoke into a December 1977 afternoon as a chain reaction of explosions destroyed this export elevator at Westwego, LA. (PN-4182)



Above: Coating grain with vegetable or mineral oils is one way to reduce dust emissions in grain elevators. In studies at the Grain Marketing Research lab, agricultural engineer Charles Martin uses an ultrasonic spray nozzle to apply oil to grain so dust will stick to kernels and not become airborne. (0985X 1049-18)

Facing page, left: A high-speed motion picture camera records kernel breakage at 1,300 frames per second as they fall onto a pile of grain. (PN-7190)

Facing page, top: Using a laboratory grain accelerator designed at the Grain Marketing Research Laboratory, research associate Zuzanna Czuchajowska simulates 100 feet per second velocity of grain falling into an elevator. (0985X1052-18)



Technician George Wyatt operates the high-volume sampler which vacuums dusty air from the spout to simulate dust generated by the flow of grain at commercial elevators. (0785X635-25)







Technician Dan Brabec measures grain dust emission characteristics with a drop-test system developed at the U.S. Grain Marketing Research Laboratory in Manhattan, KS. The instrument measures dust generated from grain samples dropped from a fixed height. (0985X1055-27A)

Books on Elevator Safety

The National Grain and Feed Association has published a practical guide that elevator operators can use to increase productivity and safety.

The 518-page book has 26 fully illustrated chapters written by people who are top names in grain industry engineering, design, construction, and safety.

The book covers many topics including how the results from NGFA's fire and explosion research can be applied to protect bucket elevators in existing facilities. Techniques include installing vent panels that release when explosive pressures develop and a portable device that emits a Halon extinguishing gas at the earliest stages of the explosion to halt the spread of flames.

To order "Retrofitting and Constructing Grain Elevators for Increased Productivity and Safety," send a check for \$65 payable to the National Grain & Feed Association, 725 15th St., N.W., Washington, DC 20005.

Anyone ordering this latest book can buy a 1982 NGFA book called "Dust Control for Grain Elevators" at the special discount price of \$15 while supplies last.

Also, individual reports from each of the 30 completed research projects are available at cost. Simply write to NGFA and ask for an order form.

Ion Channels in Plants Work Like Simple Nervous System



Plant physiologists Charles Mischke (rear) and Charles Bare perform patch clamping, a way to measure ion movements in a microscopic patch of cell membrane. (0985X976-13)

A recent discovery confirms what scientists have suspected for years: electrically charged atoms—ions—shuttle in and out of plant cells, as they do in animal cells, through tunnels built of protein.

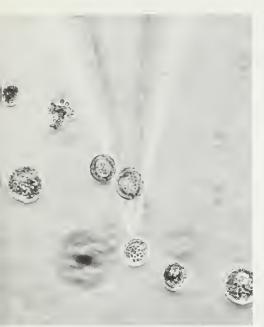
The tunnels, known as ion channels, control vital processes in plant cells and ultimately in the plant itself, says Agricultural Research Service scientist Charles F. Mischke, a member of a National Institutes of Health-U.S. Department of Agriculture team that first detected the channels in plants.

Mischke, a plant physiologist in Beltsville, MD, says the discovery will allow scientists to begin making an intelligent search for chemicals that open or block ion channels as a means to increase yields in crop plants or to control weed growth.

Each channel is a single tubelike protein—part of which acts as a gate—embedded in the membranes of plant cells. When cells are in a "resting" state, there are more positive ions outside than inside, which creates an electrical potential across the cell membrane, Mischke explains. A chemical or physical stimulus opens the channel gate, allowing specific ions to pass into or out of the cell until the two sides carry an equal charge. This depolarization of the membrane initiates a kind of electrical current—an action potential—like that in animal nerves. However, it travels about 1,000 times slower in plants than in nerves.

An action potential may radiate across many cells, for example, causing a Venus flytrap to shut down on an unsuspecting insect. Or it may stay confined to a single cell, causing no apparent reaction.

Scientists first turned to measuring action potentials in giant alga cells in the 1930's in order to understand the nature of nerve impulses. Since then, action potentials have been studied in many higher plants, but the ion channels that trigger them were not detected until recently. Progress had been impeded by the fact that plant cells are individually wrapped in a tough cel-

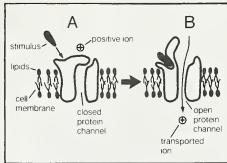


Micrograph reveals pipette sealed or clamped to patch of membrane covering wheat protoplast (cell with wall removed). Actual size of protoplast is about .0008 inch in diameter. Micrograph by Nava Moran, National Institutes of Health, Bethesda, MD. (PN-7191)

lulose wall that must be removed to get to the membrane, Mischke explains.

Mischke and Charles E. Bare of the Weed Science Laboratory at Beltsville, MD, collaborated with Nava Moran, Gerald Ehrenstein, and Kunihiko Iwasa of the National Institute of Neurological and Communicative Disorders and Stroke, NIH, who are experts in a technique called patch clamping.

According to Moran, patch clamping is a novel approach to studying cell membranes. Developed in 1976 by German scientists, it directly measures the movement of ions through individual channels in a microscopic patch of the membrane. The patch is sealed to the tip of a tiny glass pipette connected to a silver electrode. The "clamped" patch is then placed in an electrically conductive solution, which is connected to another electrode. This arrangement allows one to monitor the opening or closing of



Schematic diagram (A) shows stimulus (chemical or electrical) approaching closed tunnel, with positive ion blocked at gate. (B) Stimulus opens gate, allowing ion to enter cell.

gates that respond to the electrical or chemical signals as well as the movement of ions, she says.

At least two plant hormones and a whole class of herbicides are known to alter the permeability of cell membranes to water, and probably do so by opening or blocking ion channels. As ions move in or out of a cell, they carry water with them.

"By manipulating channels, we might increase plant growth and yield or stop growth altogether," says Mischke.

For example, in animal cells, calcium ions regulate the opening or closing of some ion channels, Mischke says, and could work similarly in plants. A private company in Virginia has greatly increased both growth and yield in a variety of crop plants with a mixture of calcium chloride and specific fatty acids, he notes. The fatty acids aid in getting calcium ions to the cell membranes where they apparently act on the ion channels.

On the other hand, says Mischke, certain ion channels are designed to shuttle calcium ions in or out of the cell. A scientific group in England found that a drug that blocks calcium channels in humans also halted the growth of pea plants.

The medical profession routinely uses ion channel blockers, such as drugs that regulate heart rhythm. And the well-known tranquilizer Valium promotes channel opening.

Manipulating ion channels in plants is a long way off, however. "We know so little about the function of ion channels in animals," he says, "and nothing about their function in plants."

Moran says that the first step will be to find what life processes are dependent on these channels. The simplest way is to block the channels and see what happens, she says. Patch clamping will enable scientists to find blockers for each type of ion channel. "No other technique allows one to 'see' the behavior of a single ion channel under various conditions," she says.

Some chemicals block ion channels like a cork in a bottle. Others bind to the gate portion of the protein to keep the channel closed or open longer than normal. There is a dynamic and delicate balance between the open and closed states of ion channels, Moran says.

The NIH-USDA team identified channels for four different ions in wheat and potato leaf cells and in carrot cells in tissue culture.

Together with Ruth Satter of the University of Connecticut at Storrs, they have found channels for two different ions in specialized cells from the tropical monkey pod tree (Samanea saman), whose leaves open and close in response to light.

The specialized cells, called pulvini, work in opposition to control leaf opening and closing similar to the action of extensor and flexor muscles in animals, Mischke explains. Daylight triggers ion channels in the extensors to open, allowing an influx of water which opens the leaves. Darkness causes the flexors to swell, closing the leaves.

Two other scientific teams in Germany and in France have confirmed the existence of ion channels in other types of plants.—Judy McBride, Beltsville, MD

Charles F. Mischke and Charles E. Bare are at the USDA-ARS Weed Science Laboratory, Rm. 24, Bldg. 001, Beltsville Agricultural Research Center-West, Beltsville, MD. 20705.

Technician Ed Bowers checks for *Aspergillus flavus* infection. The capricious occurrences of *A. flavus* in corn fields complicates tracking it. In routine surveys, only a limited number of ears in a field contain the aflatoxin-producing mold and only a fraction of the kernels of each contaminated ear are internally infected. (0885X886-22)

Aflatoxin—No One Solution

This summer's drought and the related aflatoxin outbreak that reduced North Carolina's corn crop by more than 22 million bushels is a reminder that aflatoxin has become an increasing threat in the past 20 years.

Aflatoxin is a natural poison produced by several different molds, most commonly Aspergillus flavus. When the mold invades a crop and produces aflatoxin, it makes it unsalable. Food and Drug Administration guidelines say grain with 20 parts per billion (ppb) or more should not be sold for human consumption or in interstate commerce. The FDA guideline for total aflatoxins in cornmeal, grits, and peanut butter is also 20 ppb, but for milk it is 0.5 ppb.

The FDA has been in the middle of the often turbulent aflatoxin debate, a debate that has seen much litigation. In fact, FDA has asked the Supreme Court of the United States to review a court decision that says FDA must set formal aflatoxin tolerance levels—as opposed to the current guidelines—with no exceptions.

Eivind B. Lillehoj, a microbiologist with the ARS Southern Regional Research Center in New Orleans, LA, says, "Each FDA seizure of a major aflatoxin-contaminated food or feed causes ripples in the marketing and research system."

It was a food seizure of white corn in 1971 in Missouri that led Lillehoj and colleagues at both the New Orleans lab and the ARS North Central Regional Research Center in Peoria, IL, to discover that aflatoxin-producing molds can invade corn in the field, before harvest and storage. At about the same time, scientists from the Quaker Oats Company reached similar conclusions from a study in Georgia.

Previously, it was thought that the alfatoxin-producing molds only lived on dead or decaying plants.

Turning their attention to crops in the fields, ARS researchers have found that crops under stress—such as water deprivation caused by a drought—are more susceptible to

molds, presumably because their defenses are weakened.

Farming Practices Can Stress Crops

Lillehoj says changes in farming methods in the past two decades may be increasing chances for stress on plants and increasing the number of aflatoxin outbreaks.

For example, the push for higher crop yields has caused farmers to plant more densely, increasing competition for water and the risk of water stress.

A reduced genetic diversity within varieties and large continuous acreages of the same variety may also be making plants more susceptible to stress from disease and insect pests. Also, molds are spread by insects, among other means.

As a microbiologist, Lillehoj worries about the side effects of breeding crops resistant to fungi such as the corn smut fungus. He says the resulting elimination of corn smut removed competition and may have allowed "opportunists" such as *A. flavus* to take its place.

Lillehoj says, "That's one example of why breeders must consider the whole picture, especially when it comes to breeding for aflatoxin resistance. Sometimes changing one factor in a plant can have unexpected side effects."

Despite the inherent difficulties, Lillehoj believes that breeding is one of the best ways to control the aflatoxin problem.

Researchers have already examined numerous standard inbred lines of corn (plants from which commercial hybrids are produced) in extensive tests and found some that are especially resistant to aflatoxin, at least under some conditions. But Lillehoj says many more tests of more plants in more locations will have to be done to see if the pattern of resistance in these plants holds under a variety of conditions.

Lillehoj says breeding for resistance to aflatoxin will be one of the most difficult tasks breeders have ever faced because *A. flavus* is an unusual pathogen. While one field

may be infested with the toxin, an adjoining field could have none.

The fact that aflatoxin-producing molds are opportunists that would as soon attack cottonseed or peanuts as corn makes the task much more difficult than if there had been a close host-pathogen relationship, with a gene-for-gene relationship developed over the years.

Until a strong resistance to aflatoxin is developed in crops, farmers can take advantage of what resistance may already be available. For example, Lillehoj says researchers have found that the most productive hybrids adapted to a particular area are also the most aflatoxin-resistant.

In addition to choosing the right variety for their areas, farmers should follow other good practices, such as proper control of weeds, insects, and diseases, and irrigation scheduling. These methods ensure vigorous plants less vulnerable to stress and help limit the spread of the mold. It's also critical to harvest the crop at the right moisture level and to dry it quickly to prevent aflatoxin contamination after harvest.

Predicting Aflatoxin Outbreaks

Though aflatoxin outbreaks can't be entirely prevented or controlled, they can be predicted. Lillehoj led a cooperative effort in 1980 that proved that state field trials of hybrid corn varieties could be used as early warning test plots. Experiment stations conduct state field trials throughout corn-growing regions every year to compare yields of different hybrids.

These tests provide an opportunity for researchers to study various diseases under conditions that accurately reflect what farmers face in different corn-growing areas.

Lillehoj and colleagues at North Carolina State University and the University of Missouri planted 30 hybrid corn varieties at experiment station sites in Florence and Blackville, SC. Some of the varieties were very susceptible to aflatoxin. These tests were not done as part of state field trials, but they were done in the



Microbiologist Amy Henderberg holds petri dish containing a mold transferred from corn kernels initially suspected of *A. flavus* contamination. Petri dishes are examined under ultraviolet light for a characteristic fluorescence associated with aflatoxin. (0885X885-35)

same area where the field trials are held.

The thinking was that if conditions were right for an aflatoxin outbreak in the area, the susceptible plants would be among those showing the signs first. If resistant plants began showing signs, too, then farmers could expect problems in their fields.

First, the researchers inoculated the plants with A. flavus. As kernels formed, they used a black light to check for aflatoxin in crushed kernels, looking for a bright, greenishyellow glow that is associated with the toxin.

They checked the accuracy of this field test against their laboratory assays for the actual toxin and found the results comparable, as long as the field test was done correctly. They also compared the extent of aflatoxin contamination after harvest with the damage estimated before harvest and found their predictions quite accurate.

Predicting aflatoxin contamination gives farmers an early start on making decisions such as whether to sell the crop or decontaminate the corn and feed it only to mature, nondairy ruminants or mature swine.

In the early seventies the Southern Regional Research Laboratory began experimenting with decontaminating cottonseed meal with anhydrous ammonia. By the late seventies, the procedure was simplified and adapted to corn by the North Central Regional Research Center. Lillehoi says it has emerged as the method of choice since it can be used on the farm and is relatively inexpensive. There are commercial variations of the method in use in different parts of the country. The basic idea is to decontaminate the crop by exposing it to ammonia gas in a sealed building.

While decontamination techniques evolve and breeders keep searching, farmers can only manage as they always have, with one eye on the weather and the other on their crops.—Neal Duncan, formerly at New Orleans, LA, and Don Comis, Beltsville, MD.

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PATENTS

Herbicide-Coated Seeds

Applying herbicides on or next to seeds while planting saves time and may not harm the seeds.

Field and greenhouse experiments have shown that weeds can be controlled selectively in field beans by applying the herbicide EPTC (ethyl dipropylthiocarbamate) directly to the seed before planting or as granules placed near the seeds as they are planted.

The inventor has also developed practical methods for seeding alfalfa and applying EPTC in one operation, placing the herbicide near the seed.

Limited greenhouse trials indicate that tomatoes, turnips, sunflowers, flax, and sugarbeets might also respond favorably to herbicidecoated seeds.

The amount of herbicide placed on each seed depends on the total amount of herbicide to be used, the seed spacing, and the distance the herbicide is expected to spread from the seed.

For technical information, contact Jean H. Dawson, USDA-ARS, Irrigated Agriculture Research and Extension Center, P.O. Box 30, Prosser, WA 99350. Patent No. 4,272,920, "Method of Applying Herbicide."

Pheromone for Grain Weevils

Grain weevils can now be added to the list of economically important insects that can be monitored or controlled by pheromones (sex attractants).

A pheromone produced by male rice and maize weevils has been identified, isolated, and successfully synthesized. The pheromone attracts male and female rice, maize, and granary weevils, even though granary weevils apparently do not produce the pheromone.

For technical information, contact Wendell E. Burkholder, USDA-ARS, Bee Management and Entomology Research, Department of Entomology, University of Wisconsin, Madison, WI 53706. Patent Application Serial No. 06/716,798, "Synthetic Pheromone 5-Hydroxy-4-Methyl-3-Heptanone and Its Use in Controlling Grain Weevils."

Bacterial Protein Extraction Process

Now there is a simpler and quicker way for scientists to isolate protein from bacteria for analyses.

In this method, already in use in some laboratories, bacterial cells are treated with an organic solvent and collected by centrifugation, volatile residues are removed, and the proteins are extracted with a water-based solvent.

Experimental evidence shows

this technique does as good a job as other methods, such as ultrasonic disruption and agitation with glass beads. It has the advantages of not needing special equipment, not contaminating samples with extraneous proteins, and being effective with a variety of bacterial species, including pathogens.

For technical information, contact Saumya Bhaduri, USDA-ARS, Microbiological Safety Research, 600 East Mermaid Lane, Philadelphia, PA 19118. Patent No. 4,464,295, "Simple and Rapid Method for Extraction of Proteins from Bacteria."

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